

DEC 22 2006

Application No. 10/049,975  
Filed: October 1, 2002  
Confirmation No.: 6238  
TC Art Unit: 1641AMENDMENTS TO THE CLAIMS

1. (Currently Amended) A method of assaying substances comprising the steps:

providing a surface that has a top and a bottom and at least one reaction partner R1 bonded to a the top of said surface;

placing in contact with said surface a solution that contains at least the substance being assayed, at least one compound containing a fluorophor and at least one dye that absorbs in the absorption and/or emission range of the fluorophor, wherein a complex forms on reaction partner R1 on said surface and wherein said complex is formed by covalent or non-covalent interactions of reaction partner R1, with the substance being assayed and by covalent or non-covalent interactions of the at least one compound containing at least one a fluorophor with the substance being assayed;

projecting a beam of light onto the bottom of the surface, said beam of light being totally reflected on the surface of the phase boundary, thereby forming an evanescence field over said surface, wherein said dye is present in the solution at a concentration sufficient to absorb 70% or more of the light entering the solution within 1 mm above the top of said surface; and

exciting the fluorophor bonded to said surface by the said evanescence field of a light source and measuring the fluorescence produced as a measure of the substance being assayed.

Application No. 10/049,975  
Filed: October 1, 2002  
Confirmation No.: 6238  
TC Art Unit: 1641

2. (Previously Presented) The method according to Claim 1, wherein the substance being assayed, bonds to reaction partner R1 on said surface as reaction partner R2.

3. (Previously Presented) The method according to Claim 2, wherein the reaction partner R1 bonded to said surface is an antigen or an antibody.

4. (Previously Presented) The method according to Claim 1, wherein a reaction partner R2 contains the substance being assayed and bonds to reaction partner R1 on said surface along with said substance being assayed.

5. (Previously Presented) The method according to Claim 1, wherein another compound, which contains a bonding site for the substance being assayed and a reaction partner R2, bonds to reaction partner R1 on the surface.

6. (Previously Presented) The method according to Claim 5, wherein reaction partner R1 includes avidin or streptavidin and reaction partner R2 includes biotin and a binding site for the substance being assayed.

7-26. (Cancelled)

27. (Previously Presented) The method according to claim 1, wherein the substance being assayed includes a biologically active substance, which is selected from the group consisting of hormones, proteins, viruses, bacteria, pharmaceuticals and toxins.

Application No. 10/049,975

Filed: October 1, 2002

Confirmation No.: 6238

TC Art Unit: 1641

28. (Previously Presented) The method according to claim 1, wherein the substance being assayed is a protein.

29. (Previously Presented) The method according to claim 1, wherein the compound containing a fluorophor further contains a binding site for the substance being assayed.

30. (Previously Presented) The method according to claim 1, wherein fluorescing proteins and/or low-molecular weight fluorescing chemical compounds are used as the fluorophor.

31. (Previously Presented) The method according to claim 30, wherein phycobili proteins are used as fluorescing proteins.

32. (Previously Presented) The method according to claim 31, wherein 5-N-N'-diethyltetramethylindodicarbocyanine (Cy5) or dipyrromethene boron difluoride (BODIPY) are used as low-molecular weight fluorescing compounds.

33. (Previously Presented) The method according to claim 1, wherein at least one fluorophor that absorbs in a wavelength range from 600 to 700 nm is used.

34. (Previously Presented) The method according to claim 1, wherein at least one phosphorescing compound is used as the fluorophor.

**BEST AVAILABLE COPY**

Application No. 10/049,975

Filed: October 1, 2002

Confirmation No.: 6238

TC Art Unit: 1641

35. (Previously Presented) The method according to claim 1, wherein a mixture of dyes that absorb in the absorption and/or emission range of the fluorophor is used.

36. (Previously Presented) The method according to claim 1, wherein at least one dye that absorbs in a wavelength range from 600 to 700 nm is used.

37. (Previously Presented) The method according to claim 36, wherein disodium alpha-(4-(N-ethyl-3-sulfonatobenzylamino) phenyl)-alpha-(4-N-ethyl-3-sulfonatobenzylamino, cyclohexa-2,5-dienylidene) toluene-2-sulfonate (Brilliant Blue FCF) in a concentration of at least 0.001 mM is used as the at least one dye.

38-44. (Cancelled)

45. (Previously Presented) The method according to claim 1, further comprising the step of determining reaction kinetics of immunologic reactions.

46. (Previously Presented) The method according to claim 1, further comprising the step of carrying out an assay selected from the group consisting of medical or veterinary medical diagnostics, food analysis, environmental analysis or analysis of fermentation processes.

47. (Previously Presented) The method according to claim 27, wherein:

-5-

WEINGARTEN, SCHURGIN,  
GAGNEBIN & LEBOVICI LLP  
TEL. (617) 542-2290  
FAX. (617) 451-0313

**BEST AVAILABLE COPY**

Application No. 10/049,975

Filed: October 1, 2002

Confirmation No.: 6238

TC Art Unit: 1641

the substance being assayed is a protein;

the compound containing fluorophor further contains a binding site for the substance being assayed;

fluorescing proteins and/or low-molecular weight fluorescing chemical compounds are used as the fluorophor;

phycobili proteins are used as fluorescing proteins;

a mixture of dyes that absorb in the absorption and/or emission range of the fluorophor is used; and

at least one dye that absorbs in a wavelength range from 600 to 700 nm is used.

48-51. (Cancelled)

52. (Previously Presented) The method according to claim 47, further comprising the steps of determining reaction kinetics of immunologic reactions.

53. (Cancelled)

54. (Previously Presented) The method according to claim 47, further comprising the steps of carrying out an assay selected from the group consisting of medical or veterinary medical diagnostics, food analysis, environmental analysis or analysis of fermentation processes.

55. (Cancelled)

56. (Previously Presented) The method according to claim 28, wherein the protein is an antigen or an antibody.

Application No. 10/049,975  
Filed: October 1, 2002  
Confirmation No.: 6238  
TC Art Unit: 1641

57. (Previously Presented) The method according to claim 31, wherein the phycobili proteins are selected from the group consisting of allophycocyanine (APC) and low-molecular weight cryptomonad-derived phycobili proteins.

58. (Previously Presented) The method according to claim 47, wherein the protein is an antigen or an antibody.

59. (Previously Presented) The method according to claim 47, wherein the phycobili proteins are selected from the group consisting of allophycocyanine (APC) and low-molecular weight cryptomonad-derived phycobili proteins.

60. (Previously Presented) The method according to claim 47, wherein Cy5 or BODIPY are used as low-molecular weight fluorescing compounds.

61. (Previously Presented) The method according to claim 47, wherein a fluorophor that absorbs in a wavelength range from 600 to 700 nm is used.

62. (Previously Presented) The method according to claim 47, wherein at least one phosphorescing compound is used as the fluorophor.

WEINGARTEN, SCHURGIN,  
GAGNEBIN & LEBOVICI LLP  
TEL. (617) 542-2290  
FAX. (617) 451-0313

BEST AVAILABLE COPY